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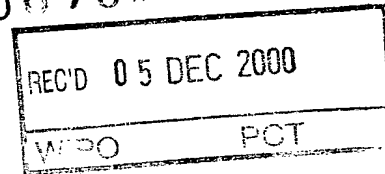
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## DESCRIPTION

In the fields of medical-, dietary-, environmental- and chemical sciences there is an increasing need for the selective separations of specific substances in complex mixtures of related substances. The end goal can be the preparative isolation of a certain compound or compounds or measurements of their concentration. Molecularly imprinted polymers (MIPs) exhibit often a high selectivity towards their substrate in analogy the antibody-antigen complementarity.(1, 2) The technique shows promise in chiral separations of for example amino acid derivatives, peptides, phosphonates, aminoalcohols and beta-blocking compounds, affinity chromatography of nucleotides and the DNA-bases as well as substitute for antibodies in immunoassays for commercial drugs. Molecular imprinting (MI) consists of the following key steps: (1) Functional monomers are allowed to interact reversibly with a template molecule in solution. (2) The hereby formed template assemblies are copolymerized with a crosslinking monomer resulting in a crosslinked network polymer. (3) The template is displaced and the materials can be used for selective molecular recognition of the corresponding compound. If these are crushed and sieved they can be packed in a chromatographic column and used for chromatographic separation of the template from structurally related analogs. Analytical as well as preparative applications are here possible. Preparative applications can be separation of a compound from a complex mixture of structurally related compounds and isolation of the compound. This can be through an affinity chromatographic procedure where pH, ion strength or solvent gradients can be used in order to control the strength of interaction with the stationary phase. The separation can target enantiomers or diastereomers in a mixture of enantiomers or diastereomers of one or many compounds. Analytical applications can in addition to the above mentioned separations be: competitive binding assays, chemical sensors or selective sample enrichments.

Currently the most widely applied technique to generate molecularly imprinted binding sites is represented by the noncovalent route developed by the group of Mosbach(3). This makes use of noncovalent self-assembly of the template with functional monomers prior to polymerization, free radical polymerization with a crosslinking monomer and then template extraction followed by rebinding by noncovalent interactions. Although the preparation of a MIP by this method is technically simple it relies on the success of stabilisation of the relatively weak interactions between the template and the functional monomers. Stable monomer-template assemblies will in turn lead to a larger concentration of high affinity binding sites in the resulting polymer. The materials can be synthesized in any standard equipped laboratory in a relatively short time and some of the MIPs exhibit binding affinities and selectivities in the order of those exhibited by antibodies towards their

antigens. Most MIPs are synthesized by free radical polymerization of functional monounsaturated (vinyl, acrylic, methacrylic) monomers and an excess of crosslinking di- or tri- unsaturated (vinyl, acrylic, methacrylic) monomers resulting in porous organic network materials. These polymerizations have the advantage of being relatively robust allowing polymers to be prepared in high yield using different solvents (aqueous or organic) and at different temperatures (4) This is necessary in view of the varying solubilities of the template molecules.

The most successful noncovalent imprinting systems are based on commodity acrylic or methacrylic monomers, such as methacrylic acid (MAA), crosslinked with ethyleneglycol dimethacrylate (EDMA). Initially, derivatives of amino acid enantiomers were used as templates for the preparation of imprinted stationary phases for chiral separations (MICSPs) but this system has proven generally applicable to the imprinting of templates allowing hydrogen bonding or electrostatic interactions to develop with MAA.(5, 6) The procedure applied to the imprinting with L-phenylalanine anilide (L-PA) is outlined in Figure 1. In the first step, the template (L-PA), the functional monomer (MAA) and the crosslinking monomer (EDMA) are dissolved in a poorly hydrogen bonding solvent (diluent) of low to medium polarity. The free radical polymerization is then initiated with an azo initiator, commonly azo-N,N'-bis-isobutyronitrile (AIBN) either by photochemical homolysis below room temperature(6, 7) or thermochemically at 60°C or higher(5). Lower thermochemical initiation temperatures down to 40°C or 30 °C may be obtained using azo-N,N'-bis-divaleronitrile (ABDV) and V70 resp. instead of AIBN as initiator (see chapter 3).(7, 8) In the final step, the resultant polymer is crushed by mortar and pestle or in a ball mill, extracted by a Soxhlet apparatus, and sieved to a particle size suitable for chromatographic (25-38 µm) or batch (150-250 µm) applications.(6) The polymers are then evaluated as stationary phases in chromatography by comparing the retention time or capacity factor ( $k'$ )(9) of the template with that of structurally related analogs.

As appears from above MIPs have so far been prepared in the form of continuous blocks that need to be crushed and sieved before use. This results in a low yield of irregular particles, a high consumption of template and a material exhibiting low chromatographic efficiency. There is therefore a need for MI-materials that can be prepared in high yield in the form of regularly shaped particles with low size dispersity and a controlled porosity. These are expected to be superior in terms of mass transfer characteristics and sample load capacity compared to the materials obtained from the monolith approach.

Such MIPs have been previously prepared through suspension(10, 11)- polymerisation techniques, dispersion polymerization(12) or precipitation polymerization(13). This resulted in spherical particles of a narrow size distribution. These procedures have the limitation of being very sensitive to small changes in the manufacturing conditions and the type of solvents and

polymerization conditions that can be applied. Thus the procedures need careful optimization for each new template target which significantly reduces the usefulness of this route. Moreover conditions leading to lowdispersity spherical particles may not be compatible with conditions leading to high selectivity and affinity for the template target.

An alternative to this procedure is the coating of preformed support materials.(14-16) MIPs have been prepared as grafted coatings on metaloxide supports(14, 16) on organic polymer supports(15) and on the walls of fused silica capillaries(17-19). The former technique allows the use of the wide variety of metal oxide support materials available with different sizes and porosities. Grafting techniques to prepare organic polymer coatings are expected to be generally applicable to molecular imprinting since the structure of the underlying support is already fixed. Thus compared to the large number of factors influencing the end result in suspension or precipitation type polymerizations a smaller number of factors is likely to influence the end result in the preparation of the imprinted coatings. This will make the grafted coatings techniques less sensitive to changes in conditions offering a more robust method. This type of coating techniques are furthermore applicable to modify surfaces of monolithic type supports or microchips prepared by lithographic techniques. The metaloxide based materials are rigid porous supports with a limited inner pore volume. An alternative support that could potentially carry more grafted imprinted polymer per unit weight and thus allow a higher density of imprinted sites would be to make use of swellable organic resins. In this context Merrifield resins containing grafted initiator or monomer could be used.

Sofar most imprinted coatings have been prepared by grafting polymers to the various surfaces. Thus the surface contains prior to polymerization polymerizable double bonds that can add to the growing polymer chains in solution linking them to the surface. The problem with this technique is the presence of initiator in solution requiring the monomer mixture to be applied as a liquid thin film on the surface prior to polymerization. Thus the exact amount of monomers that will coat the available surface with an up to ca 100 Å thick liquid film are dissolved together with initiator in an excess of solvent. Thereafter the modified support is added and the solvent evaporated to leave the monomer film and initiator on the surface. Polymerization is then carried out usually at elevated temperatures. With this procedure the thickness of the polymer layer is difficult to control and capillary forces upon evaporation of solvent may cause incomplete wetting of the surface. Moreover a continuous method of synthesising the particles is difficult to envisage with this method.

A considerable improvement in this regard would be to confine the initiator radicals to the support surface (Figure 2).(20, 21) In absence of chain transfer this would lead to chain growth occuring only from the surface of the support with no polymerization occuring in solution. For molecular

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imprinting this would have important consequences. For instance the polymerization can be carried out the surface of initiator modified support particles suspended in a mixture of the monomers and solvent. This would allow polymerization in a simple tank reactor by either thermal or photochemical initiation. The latter technique would allow the particles to be modified during the sedimentation possibly leading to a continuous method for preparing the imprinted composite particles (Figure 3). Polymerization would here only occur on the particle surface leaving the solution containing the monomers unreacted. The monomer solution can thus be reused for the coating of several batches of particles. The problem of confining polymer chain growth to the support surface and suppress it in solution can be solved by attaching the radical initiator so that the radical formed upon bond homolysis remain bound to the surface. Alternatively the radical formed that is not attached to the surface should undergo rapid reaction to give an unreactive species. It should be possible to prepare the grafted coatings using monomers such as those based on styren/divinylbenzene, methacrylates, acrylates, acrylamides and in the presence of one or more template molecules.

The invention will now be described in more detail with reference to a number of non-limiting examples:

The invention refers to a material coated with a polymer layer, a method for its fabrication and use of named material in for instance chromatography, for separations, in chemical sensors, in selective sample enrichment, in molecular recognition as stationary phase in capillaries or in catalysis. The material is prepared by grafting a polymer layer on the surface of a preformed organic or inorganic support material or surface. The grafting can be combined with technique of molecular imprinting.

The support surface is prepared as follows. A free radical initiator is bound to the surface either covalently or noncovalently so that the free radicals generated upon initiation remains confined to the surface or vicinity of the surface. The absence of polymer propagation in solution will lead to a higher accessibility of the monomers at the surface. Furthermore this method will allow the tuning of the thickness of the polymer layer.

#### *Surface attachment of free radical initiator*

This method was introduced by Guyot et.al. (21) and Tsubokawa et.al.(22, 23) It relies on presilanization of the surface using 3-aminopropyltriethoxysilane or a glycidoxypropylsilane (GPS) followed by reaction of the aminogroups or the epoxy groups with an azoinitiator such as azo-bis(cyanopentanoic acid, ACPA) leading to the formation of an amide (using DCC as condensing reagent) or ester link between the surface and the azoinitiator. Also peroxyinitiators

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may be used although better results are obtained using the grafted azoinitiator followed by photochemical initiation. High yields of grafted polymer is obtained using silica reacted with toluene-2,4-diisocyanate (TDI) followed by reaction with ACPA.

*Example 1 Coupling of initiator to amino, epoxy or chloromethyl modified supports or resins*

*Epoxy and chloromethyl modified supports:* A typical example is as follows. Into a flask, 3 g of epoxymodified particles 50 mL of DMSO, 0.5 g of ACPA and picoline were charged. The reaction mixture was stirred for 5 h at 50 °C. After the reaction the particles were washed with methanol and dried.

*Aminomodified supports:* A typical example is as follows. Into a flask, 3 g of epoxymodified particles 50 mL of DMF, 0.5 g of ACPA and dicyclohexyldicarbodiimide (DCCI) and base were charged. The reaction mixture was stirred for 5 h. After the reaction the particles were washed with methanol and dried.

The above procedure do not confine all initiator radicals to the surface since the initiator is bound at only one position. This invention describes three alternative procedures to confine the polymerization to the surface.

1. The use of a presynthesized azosilane (Figure 4A). This will more likely lead to a two point attachment of the initiator to the surface.

*Example 2 Synthesis of azosilane for two point coupling of azoinitiator to surface or support*

The azosilane was synthesized by mixing 0.5 mole glycidoxypyrroltrimethoxysilane (GPS) and 0.25 mole ACPA in 200 mL isopropanol and catalytic amounts of picoline. The reaction was allowed to continue at room temperature and the product isolated by evaporation to dryness followed by purification by column chromatography giving the product in 60 % yield.

*Example 3 Coupling of silane to surface*

The silane was coupled by reaction in water at low temperature < 20 °C for 24 hours.

2. Preadsorption of an initiator that is insoluble in the solution containing monomer. Thus a polar water soluble initiator as for instance an azo-bis-amidine, (24) can be adsorbed to the surface from aqueous solvent, the surface dried and then the polymerization initiated as above described (Figure 4B). The free radicals generated from the initiator will stay associated to the surface due to their insolubility in the monomer mixture.



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*Example 4 Adsorption of amidineazoinitiator to support surface*

An amidineazoinitiator such as 2,2'-azobis(N,N'-dimethyleisobutyramidine) or 2,2'-azobis(2-arnidinopropane) is dissolved in methanol/water and supportparticles such as silica charged. After several hours equilibration the solvent is removed by filtration and the particles dried under vacuum.

3. Use of microwaves to selectively heat the particle surface (Figure 4C).

*Example 5 Microwave initiated polymerization*

Particles is charged to a solution of monomers and initiator in a suitable solvent. The polymerization is initiated by microwaveirradiation at a wavelength causing local heating of particles only.

4. Use of iniferters such as dithiocarbamate coupled onto the surface (Figure 4D).(25)

*Example 6 Synthesis of support or polymeresin bound initiator*

To a surface or polymer containing bound chloromethyl groups is given N,N-dithyldithiocarbamate in solution and the reaction allowed to proceed at elevated temperatures.

*Example 7 Synthesis of block-graft imprinted copolymer*

Particles or a surface containing bound dithiocarbamate groups is added to a mixture of monomers (concentration ca 5 mol/L), template and solvent under nitrogen. The polymer was initiated by irradiation with a ultrahighpressure mercury UV lamp and allowed to proceed for a certain time. Then the unreacted monomers and template were washed away. The obtained particles or surface can then be immersed in another solution containing another monomer and the procedure repeated. This allows the manufacturing of layered surfaces containing one or more imprinted layers using possibly different templates and layers of different polarity or other functional properties.

*Example 8 Encapping of unreacted silanol groups*

Prior to polymerization endcapping of unreacted silanol groups can be done. Hexamethylsilazane is here effective. Good wetting is critical for the formation of a homogenous layer fully covering the support. Another possibility to enhance the wetting is to use organosilanes containing functionalities resembling solvents known to be good solvents for the methacrylate polymerizations. Among these chlorinated hydrocarbons are particularly useful.

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***Grafting of polymer layer***

The polymerization can here be carried out in a stirred suspension of the particles in the monomer mixture since growth only takes place on the surface (see Figure 3). Thus the initiator modified particles are added to a solution containing monomer and solvent and possibly a template and the suspension stirred. The polymerization is then carried out photochemically or thermally. The particles can be based on any inorganic or organic support material and the template any molecule or ion dissolved in the monomer mixture solution. The grafting can also occur on other surfaces such as those generated by lithographic processes or on the walls of capillaries. The thickness of the polymer layer is tunable by varying the time of reaction.

*Example* To a stirred solution of 38 ml (0.2 mole) EDMA, 3.4 ml (40 mmole) MAA and 10 mmole terbutylazine (or no template) in 56 ml dichloromethane is added 5 g of any of the initiator modified particles described in Example 1-6. The suspension is sparged with nitrogen and the polymerization initiated by UV irradiation using standard mercury high pressure lamp at 15°C or by heating to a temperature providing a suitable rate of polymerization. The suspension is stirred under nitrogen and UV irradiation or heating for 24 h and the particles then filtered, washed and dried under vacuum. The monomer mixture is then used to modify a second batch of particles. The resulting particles exhibit high selectivity and affinity for the template, terbutylazine.

**CLAIMS**

1. A composite molecularly imprinted material prepared by grafting a polymer layer from a surface containing bound polymerization initiators.



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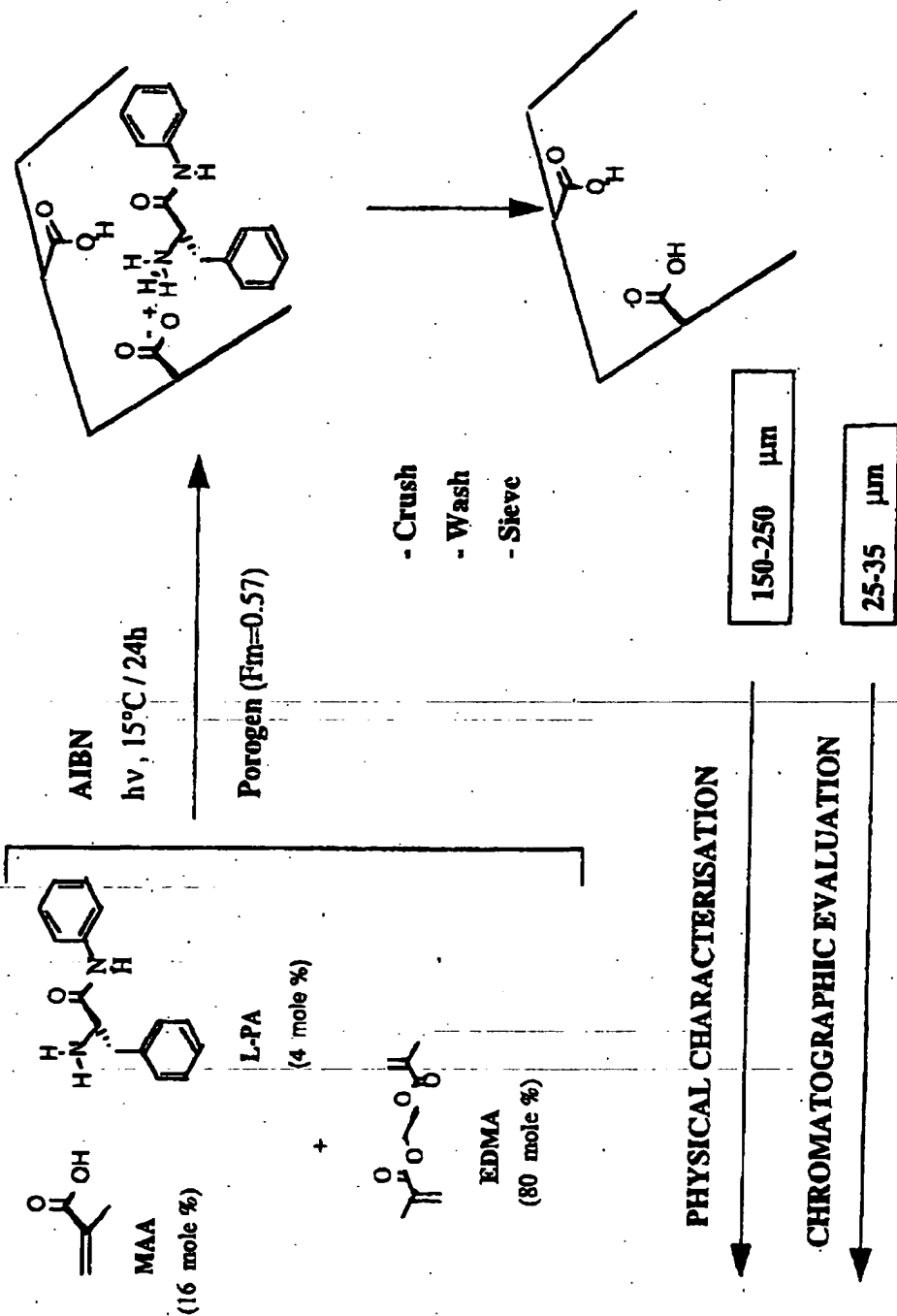
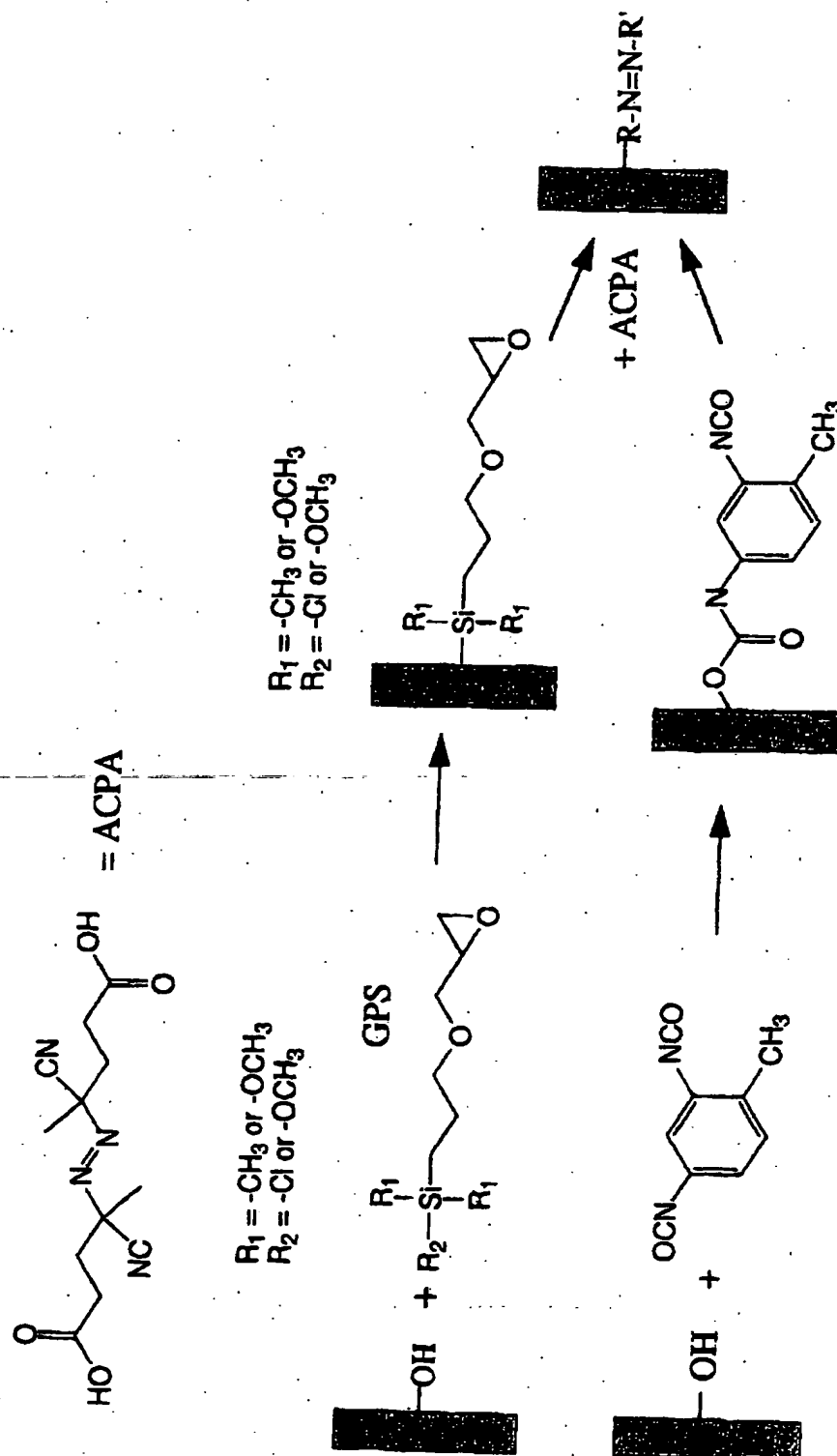


Figure 2



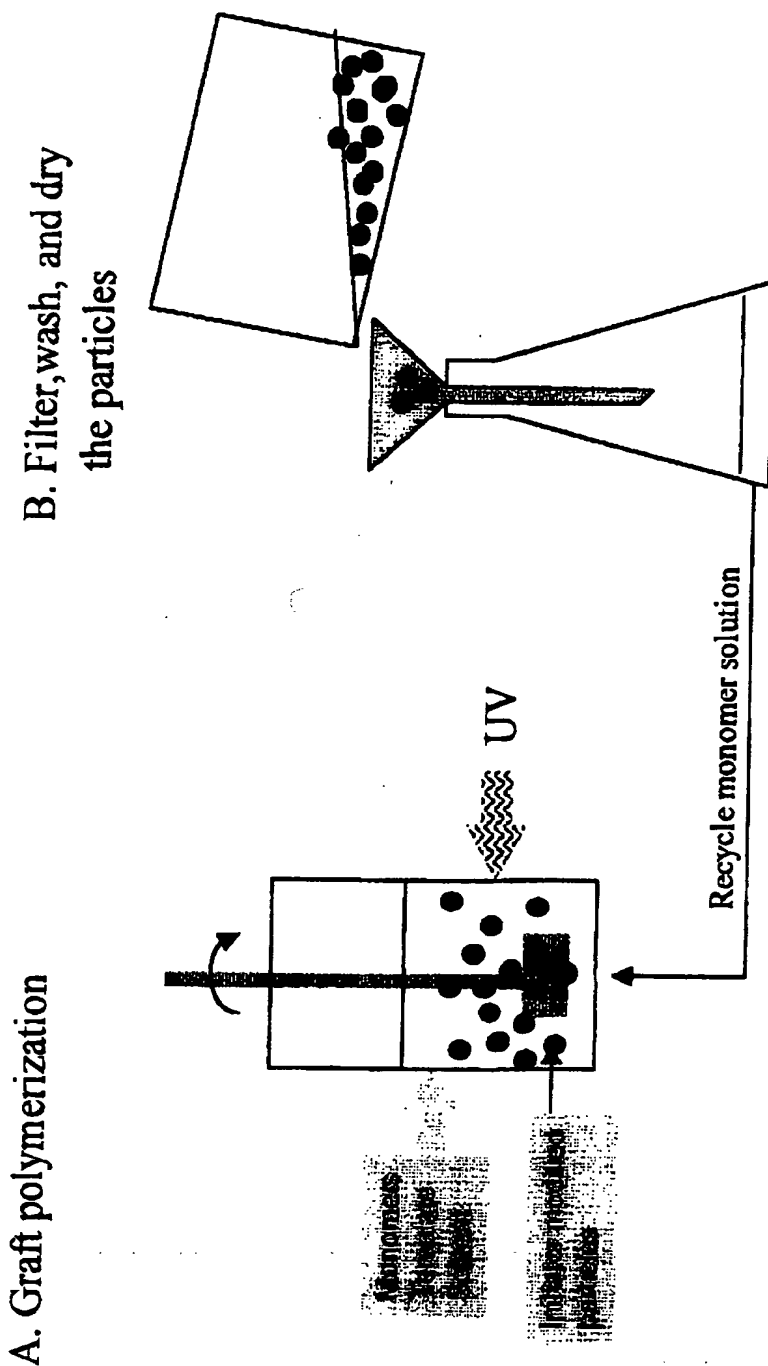
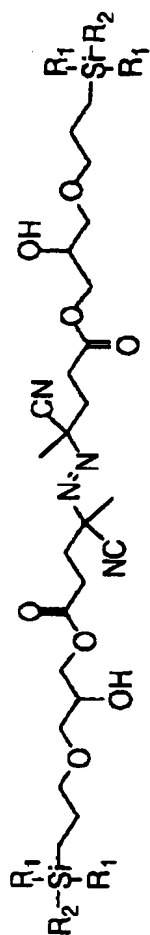


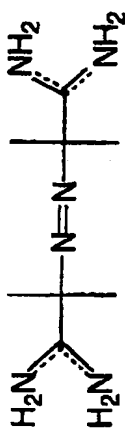
Figure 3

Figure 4

A



B



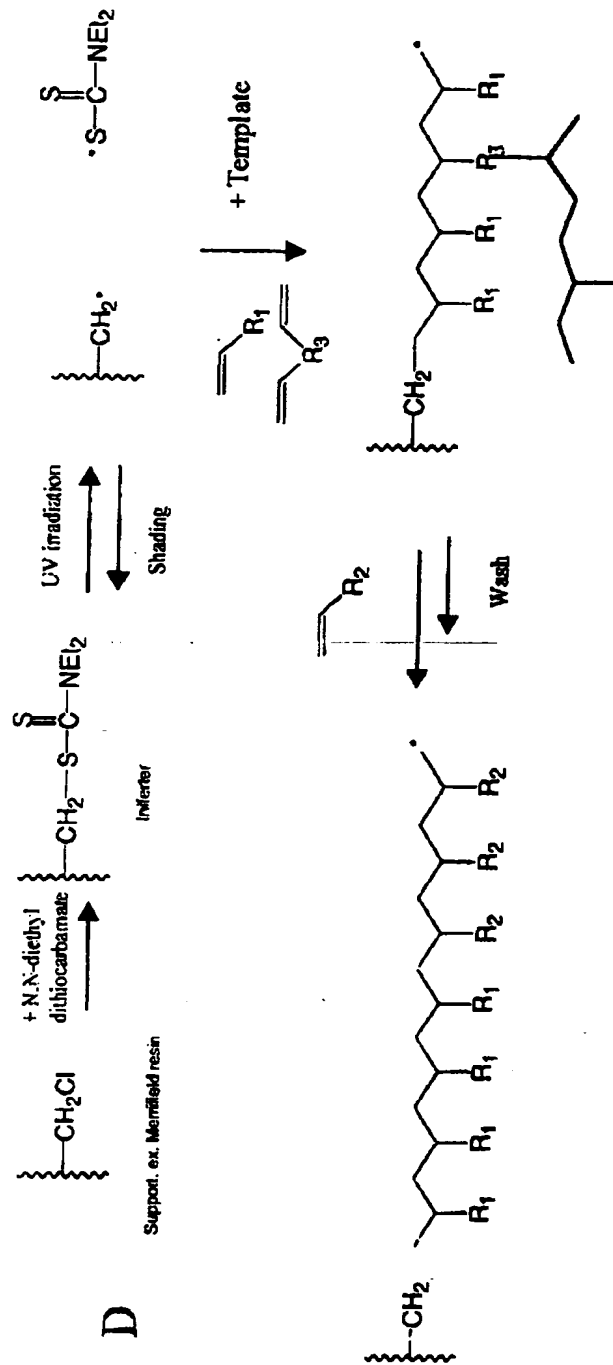
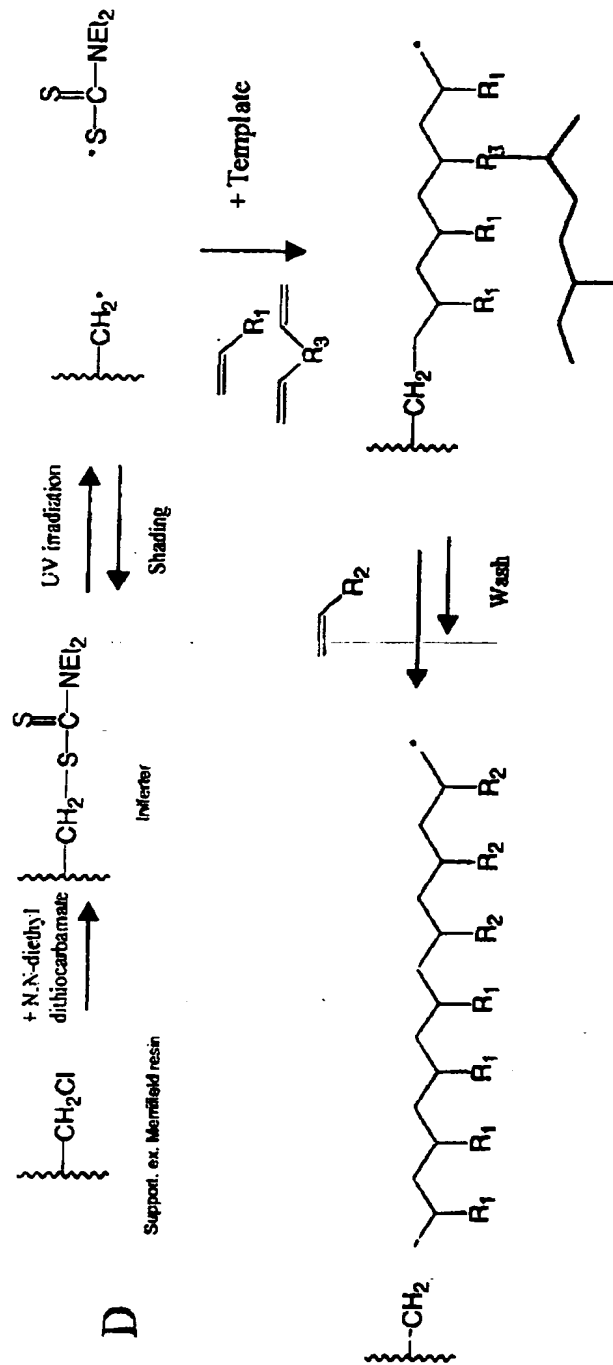
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